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(54) Title: RIBOFLAVIN AS A TANNING ENHANCER

(57) Abstract

A method of enhancing *in vivo* melanin production is disclosed. The method comprises topically applying an effective amount of riboflavin, riboflavin phosphate or mixtures thereof to the surface of the skin. Also disclosed is a composition for enhancing *in vivo* melanin production. The composition comprises an effective amount of riboflavin, riboflavin phosphate or mixtures thereof. The composition can contain at least one other ingredient selected from the group consisting of: Protein Kinase C Activators, DOPA phosphates, sunscreening agents, emollients, emulsifiers, solvents for sunscreening agents, waxes, thickeners, film formers, humectants, antioxidants, perservatives, surfactants, perfumes, biological additives, buffering agents, chelating agents, emulsion stabilizers, opacifying agents, pH adjusters, propellants and coloring agents. The Protein Kinase C Activators in the methods and compositions of this invention can be selected from the group consisting of: diacylglycerols, triacylglycerols, lipopolysaccharides, unsaturated free fatty acids, short chain saturated free fatty acids, glycerolphospholipids, enzymes which hydrolyze glycophospholipids to diacylglycerols, and bryostatins.

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RIBOFLAVIN AS A TANNING ENHANCER

REFERENCE TO RELATED APPLICATION

This application is related to my copending application Serial No. 07/434,047 filed November 9, 1989, the disclosure of which is incorporated herein by reference thereto.

FIELD

This invention relates to the enhancement of melanin production by the topical application to the skin of Riboflavin.

BACKGROUND

Melanin pigmentation is largely responsible for normal skin color and protection against ultraviolet damage, including photocarcinogenesis. Melanin is produced in melanocytes, neural crest derived cells situated in the basal layer of the epidermis, and is transferred via dendrites to surrounding keratinocytes, the most abundant cell in the epidermis. Gordon et al. disclose that this anatomical relationship, termed the epidermal melanin unit, is envisioned as one melanocyte in contact with an estimated 36 keratinocytes in the basal and suprabasal layers. According to Gordon et al., the rates of pigment synthesis and transfer by melanocytes appear to be influenced by ultraviolet light exposure and by certain inflammatory processes, but the precise factors regulating human epidermal pigmentation are unknown. Gordon et al. also disclose that it is also unknown whether these stimuli act directly on melanocytes, keratinocytes, other cells that in turn

release melanocyte mediators, or via both direct and indirect mechanisms. According to Gordon et al., the close physical contact and known functional interrelationship of the epidermal melanin unit make keratinocyte mediation of melanocyte function an attractive hypothesis. See Gordon, P.R., et al., "Regulation of Human Melanocyte Growth, Dendricity, and Melanization by Keratinocyte Derived Factors", Journal of Investigative Dermatology, 92:565-572, 1989.

According to Joshi et al., the primary events that are observed when human skin is exposed to ultraviolet radiation are immediate pigment darkening (a transient oxygen dependent photooxidation event), delayed sunburn, and tanning. Joshi et al. disclose that a wide variety of short-lived reactive oxygen species are known to be generated in skin photosensitization reactions in the presence of exogenous or endogenous photosensitizers such as riboflavin. Joshi et al. disclose an in vitro study of the role of reactive oxygen in photosensitization and tanning reaction using riboflavin (RF), hematoporphyrin (HP), 3-carbethoxypsoralen (3-CP), and 8-methoxypsoralen (8-MOP). Joshi et al. report that reactive oxygen produced by photosensitized RF, 3-CP, and 8-MOP was found to oxidize tyrosine and DOPA to dopachrome and subsequently their conversion to melanin. Joshi et al. also report that DOPA was oxidized to dopachrome and subsequently to melanin at a variable rate RF>3-CP>HP>8-MOP. According to Joshi et al., their observations appear to have relevance to the oxygen-requiring immediate tanning reaction of the skin stimulated by the UVA (320-400 NM) portion of solar radiation and in the induction of skin photosensitization. See Joshi, P.C. et al., "Involvement of Reactive Oxygen Species in the Oxidation of Tyrosine and DOPA to Melanin and In Skin Tanning," Biochemical and Biophysical Research Communications, Vol. 142, No. 1, pp. 265-274, January 15, 1987.

Those skilled in the art will recognize that there is no data presented in the in vitro study of Joshi et al. that

supports the authors inference that the in vitro photosensitization of tyrosine and DOPA to dopachrome also resulted in the subsequent conversion of dopachrome to melanin. It will be appreciated by those skilled in the art that the in vitro study of Joshi et al. relates to immediate tanning (IT) reactions, also known as immediate pigment darkening (IPD). IPD is a transient phenomenon observed in the skin after irradiation that then fades away. Immediate tanning may begin immediately and fade within seconds to a minute or may persist with higher doses or longer exposures for 1/2 to 1 hour or up to 24 hours. Rarely, IT may persist for 36-48 hours after prolonged exposure, at which stage it blends with delayed tanning (DT). See Regan, editor, The Science of Photomedicine, pp. 241-242, 1982. According to Fitzpatrick et al., IT occurs within minutes of exposure to UVA (320-400 nm) and to visible light. Fitzpatrick et al. disclose that IT becomes most prominent within 1 hour of exposure and almost completely disappears within 4 hours. Fitzpatrick et al. further disclose that studies with electron spin resonance have shown that IT reaction is probably an oxidation reaction that involves the generation of unstable semi-quinone-like free radicals in melanin. According to Fitzpatrick et al., DT develops 48-72 hours after exposure to UV light, and DT involves new production of melanosomes and therefore appears slowly over a period of days. See Fitzpatrick et al., editors, Sunlight and Man, p. 175, 1974. Thus, there is no disclosure nor suggestion relating to the in vivo effects of, for example, riboflavin on melanogenesis (tanning).

It is also known that the key steps in the biochemical pathway of melanogenesis are the hydroxylation of tyrosine to dopa (3,4-dihydroxyphenylalanine) and the oxidation of dopa to dopaquinone via the catalytic action of the enzyme tyrosinase. Duggan et al. disclose that the reactions beyond dopaquinone (see for xample their Fig. 2 on page 9, in the reference cited at the end of this paragraph) were once believed to be spontaneous but that it has been determined that these reactions are regulated by

several biological factors. According to Duggan et al., dopachrome conversion factor (DCF) increases the conversion of dopachrome to 5,6-dihydroxyindole, and 5,6-dihydroxyindole conversion factor (ICF) increases the conversion of 5,6-dihydroxyindole to the quinone and subsequently to melanin. According to Duggan et al., the desire for a deep, dark tan has generated the proliferation of cosmetic products claiming to enhance or accelerate the tanning process--e.g., Germaine Monteil's Pre Tan Starter (1981), Estee Lauder's Golden Pre-Tan Accelerator with a Bio-Tan complex (1985), and Plough's Coppertone Natural Tan Accelerator (1986). Duggan et al. further disclose that these products contain tyrosine, tyrosine derivatives, tyrosine/riboflavin complex and/or amino acid blends. According to Duggan et al., tyrosine is used to increase the substrate available for tyrosinase, and tyrosine was complexed with riboflavin in order to accelerate tyrosine's oxidation. See Duggan, M., et al., "Tyrosinase...The Enzyme Behind the Tan", Cosmetics & Toiletries, pp. 97-101, March 1987.

Consumers are becoming more and more health conscious, and as such there is increasing concern over excessive exposure to solar or artificially produced UV radiation during attempts to obtain a tanner appearance. The production of melanin is the body's response to exposure to UV radiation which results in a tanned appearance. A method and a composition for enhancing or increasing melanin production upon exposure to UV radiation which can result in a faster, darker and safer tan and would be a welcome contribution to the art. This invention provides just such a contribution.

SUMMARY OF THE INVENTION

It has surprisingly and unexpectedly been discovered that topical applications of effective amounts of riboflavin as the only active ingredient --i.e., not complexed with other substances such as tyrosine-- are sufficiently absorbed through the skin to ffect an enhanced, potentiated or increased

production of melanin upon exposure of the skin to UV radiation. This is particularly surprising, because in vitro effects of riboflavin on melanin precursors is not suggestive of in vivo enhancement of melanogenesis by riboflavin, particularly when such in vivo use is by topical applications to the skin. Thus, it has been discovered that an effective amount of riboflavin, when applied to the skin, penetrates the stratum corneum and the epidermis to reach the melanocytes. Without wishing to be bound by theory, it is believed that the absorbed riboflavin enhances, potentiates or increases the growth and replication of melanin precursors (such as tyrosinase, melanosomal proteins, and melanoprotein) upon exposure to UV radiation (UVA 320-400 nm and/or UVB 290-320 nm) through interaction with the cell membrane and/or nuclear membranes. This enhanced production of melanin precursors, enzymes and ultimately melanin itself results in more melanin being produced than would normally be produced under similar conditions but without the topical use of effective amounts of riboflavin.

Thus, this invention provides a method of enhancing melanin production comprising applying topically to the skin an effective amount of a vitamin selected from the group consisting of riboflavin, riboflavin phosphate and mixtures thereof. The riboflavin, riboflavin phosphate or mixtures thereof is applied in amounts effective to stimulate the enhanced production of melanin. Generally, the riboflavin, riboflavin phosphate or mixtures thereof is combined with suitable solvents and other optional ingredients and applied as a composition.

Another embodiment of this invention provides a topical composition for enhancing melanin production comprising an affective amount of riboflavin, riboflavin phosphate or mixtures thereof. The topical composition may optionally contain effective amounts of protein kinase C (PK-C) activators, DOPA phosphates and/or sunscreening agents.

Yet another embodiment of this invention provides a composition comprising:

(a) riboflavin, riboflavin phosphate or mixtures thereof present in an amount effective to enhance melanin production when said composition is applied topically to the skin, and

(b) a physiologically acceptable cosolvent mixture comprising water and an effective amount of a humectant.

Still another embodiment of this invention provides a composition comprising:

(a) riboflavin, riboflavin phosphate or mixtures thereof present in an amount effective to enhance melanin production when said composition is applied topically to the skin,

(b) a physiologically acceptable cosolvent mixture comprising water and an effective amount of a humectant, and

(c) optionally, at least one ingredient selected from the group consisting of: PK-C activators, DOPA phosphates, sunscreening agents, emollients, emulsifiers, solvents for sunscreening agents, solvents for said PK-C activators, waxes, thickeners, film formers, antioxidants, preservatives, surfactants, perfumes, biological additives, buffering agents, chelating agents, emulsion stabiliz rs, opacifying agents, pH adjusters, propellants, and coloring agents.

Preferably, when PK-C Activators are added to the compositions, an antioxidant is used.

DETAILED DESCRIPTION OF THE INVENTION

In general the riboflavin, riboflavin phosphate or mixtures thereof are in a concentration which is effective to provide the desired level of activity. Usually the riboflavin, riboflavin phosphate or mixtures thereof are present in an amount of at least about 0.05 percent by weight of the total composition. Generally, the riboflavin, riboflavin phosphate or mixtures thereof are present in an amount of about 0.1% to about 2% by weight of the total composition with about 0.1% to about 0.3% being preferred and about 0.15% to about 0.3% being most preferred and about 0.2% to about 0.3% being even more preferred.

Generally, the composition is applied in a sufficient amount to uniformly coat the skin. Usually the composition is applied in an amount sufficient to provide about 0.01 mg to about 0.08 mg riboflavin, riboflavin phosphate or mixture thereof to an area of skin about 10 to about 12 cm^2, with about 0.01 mg to about 0.06 mg riboflavin, riboflavin phosphate or mixture thereof being preferred and about 0.02 mg to about 0.05 mg being most preferred. Normally, the riboflavin, riboflavin phosphate or mixture thereof composition is applied at least 1 to about 6 times with about 1 to about 3 times being preferred over a time period of about 24 hours.

Conveniently, the riboflavin, riboflavin phosphate or mixtures thereof may be combined with (such as by mixing, blending or dissolving) a known formulation (vehicle) for a sunscreening agent. The inclusion of the sunscreening agent would be optional. Normally, such a formulation contains effective amounts of water and humectant.

The compositions of this invention may also be formed by combining the riboflavin, riboflavin phosphate or mixtures thereof with effective amounts of water and a

humectant. These compositions are predominantly water with enough humectant added to form a cosolvent mixture that will dissolve the riboflavin, riboflavin phosphate or mixtures thereof. Usually, in these compositions the humectant is present in amounts of about 1 to about 7% by weight of the total composition with about 4 to about 5% being preferred. The balance of the composition is water such that the total amount of ingredients (water, humectant, and riboflavin, riboflavin phosphate or mixture thereof) equals 100% by weight. Thus, such compositions may contain water in amounts of about 91 to about 98.95% by weight of the total compositions with about 91 to about 98.9% being suitable. These compositions may conveniently contain one or more of the above mentioned optional ingredients. Thus, the compositions with the optional ingredients can contain water in an amount of about 40 to about 86% by weight of the total composition, a humectant in amounts of about 1 to about 7% by weight of the total composition with about 4 to about 5% being preferred, with the balance of ingredients being selected from amongst the optional ingredients such that the total amount of ingredients (components) equals 100% by weight. The composition may be formulated by combining all the ingredients except for enough water (e.g., 5% by weight of the total composition) to make a slurry of the riboflavin, riboflavin phosphate or mixtures thereof, then the slurry is added into the composition.

Humectants well known in the art may be used. Examples of humectants include propylene glycol, sorbitol, and glycerin. Other suitable humectants may include fructose, glucose, glutamic acid, honey, maltitol, methyl gluceth-10, methyl gluceth-20, sodium lactate, sucrose, and the like.

Liposomes (lipid vesicles) may also prove useful to encapsulate the riboflavin, riboflavin phosphate or mixtures thereof. Liposomes are aqueous compartments nclosed by a lipid bilayer. They are produced by techniques well known to those skilled in the art. For example, liposomes can be produced

by suspending a suitable lipid, such as phosphatidyl choline, in an aqueous medium. This mixture is then sonicated to give a dispersion of closed vesicles that are quite uniform in size. See, for example, Stryer, Biochemistry, Third Edition, pp. 290-292, ©1988, the disclosure of which is incorporated herein by reference thereto.

Among the useful liposomes are stratum corneum lipid liposomes formed from epidermal ceramides, cholesterol, palmitic acid and cholesterol sulfate as described in Abraham et al., The Journal of Investigative Dermatology, 90, 259-262 (1988).

Many lipids are believed suitable for use in making the liposomes, many of which are commercially available, e.g. Liposome Kit is available from Sigma Chemical Company, St. Louis, Missouri under catalog number L-4262. Liposome Kit L-4262 contains L-alpha-phosphatidylcholine (egg yolk), dicetyl phosphate and cholesterol. It is a negatively charged lipsome mixture, another suitable negatively charged liposome mixture available from Sigma Chemcial Company is L-4012 which contains L-alpha-phosphatidylcholine, dicetyl phosphate and cholesterol. Suitable positively charged liposome mixtures available from Sigma Chemical Company contains L-alpha-phosphatidylcholine, stearylamine and cholesterol (catalog numbers L-4137 and L-3887).

Categories of lipids in suitable liposomes are phospholipids, glycosphingolipids, ceramides, cholesterol sulfate and neutral lipids. Various combinations of these lipids are found in neonatal mouse, pig and human stratum granulosum and stratum corneum. Other categories of lipids which can be used to make the liposomes are straight chain fatty acids, glycerol esters, glycerides, phosphoglycerides, sphingolipids, waxes, terpenes and steroids. Specific preferred lipids suitable for use are phosphatidyl choline, dicetyl phosphate and cholesterol.

The riboflavin, riboflavin phosphate or mixtures thereof can be encapsulated in (or trapped in) the compartment portion of the liposome by adding a solution of riboflavin, riboflavin phosphate or mixtures thereof and cosolvent mixture to a suitable lipid and mixing (--e.g., sonicating) to produce the liposomes containing the riboflavin, riboflavin phosphate or mixtures thereof.

The liposome can then be combined with a suitable topical vehicle, e.g. a lotion, gel or cream vehicle.

The lipid mixture which forms the liposome can be any of the conventional mixtures available or discussed in the literature which are pharmaceutically and cosmetically acceptable.

Preferred lipid mixtures contain a phosphatidyl choline, dicetyl phosphate and cholesterol. The lipid mixtures which form the liposomes are commercially available in a solvent such as ethanol or chloroform. A typical mixture contains on a weight basis, seven parts phosphatidylcholine, 2 parts dicetyl phosphate and one part cholesterol.

The compositions of this invention can contain a penetration enhancer to enhance the absorption of the riboflavin, riboflavin phosphate or mixtures thereof into the skin. The enhancer can be used in amounts of about 0.5% to about 99% by weight of the total composition, with about 1% to about 25% being preferred and about 2% to about 10% being most preferred. Representative examples of penetration enhancers include, but are not limited to: DMSO (dimethyl sulfoxide), Azone (laurocapram, 1-dodecylazacycloheptan-2-one, from Nelson Research, Irving, CA), N-methylpyrrolidone, alcohols such as panthenol, the SD alcohols and oleic alcohol, fatty acids such as oleic acid and linoleic acid, liposomes, and the like.

The compositions of this invention can contain, as stated above, PK-C activators, DOPA phosphates (such as a mixture of monophosphorylated isomers of DOPA --see U.S. Patent No. 4,508,706, the disclosure of which is incorporated

herein by reference thereto) sunscreening agents, emollients, emulsifiers, solvents for sunscreening agents, waxes, thickeners, film formers, humectants, antioxidants, preservatives, surfactants, perfumes, biological additives, buffering agents, chelating agents, emulsion stabilizers, opacifying agents, pH adjusters, propellants, coloring agents, and the like. The compositions can be formed into formulations, such as lotions, creams, gels, aerosols, and sticks, in accordance with procedures well known in the art.

The PK-C Activators useful in this invention are those Activators which are physiologically compatible with the skin, are readily absorbable through or into the skin, and penetrate through the stratum corneum and the epidermis to reach the melanocytes. The PK-C Activators may be used individually or in combination. Suitable PK-C Activators are those physiologically acceptable substances which activate protein kinase C by their direct action, or are substances which are metabolized to other substances which activate protein kinase C, or are substances which act upon other substances to produce a resulitng substance that activates protein kinase C and may include substances selected from the group consisting of: diacylglycerols; triacylglycerols; lipopolysaccharides; unsaturated free fatty acids; short chain saturated free fatty acids; glycophospholipids; enzymes which hydrolyze glycophospholipids (phosphoglycerides) to diacylglycerols such as Phospholipase C which hydrolyzes the phosphodiester bond linking the phosphorylated inositol unit to the acylated glycerol moiety to form diacylglycerol in the phosphoinositide cascade; and naturally occurring substances such as bryostatins which are naturally occurring macrocylic lactones found in bryozoa.

The acyl groups of the diacylglycerols and triacylglycerols can be unsaturated, saturated or a combination of unsaturated and saturated. Each acyl chain (group) contains at least 1 carbon atom (including the carbonyl carbon) and usually contains from about 1 to about 30 carbon atoms (including the

carbonyl carbon) with about 2 to about 24 carbon atoms being preferred and about 6 to about 20 carbon atoms being most preferred. Normally, the acyl group is derived from a naturally occurring fatty acid and the fatty acid usually contains an even number of carbon atoms and is unbranched. Although 1,2-diacyl-rac-glycerols are useful, the diacylglycerols are preferably 1,2-diacylglycerols, and most preferably 1,2-diacyl-sn-glycols.

Representative saturated free fatty acids (fatty acids) from which the acyl groups may be derived from include, but are not limited to: methanoic (formic); ethanoic (acetic); propanoic (propionic); butanoic (butyric); pentanoic (valeric); hexanoic (caproic); heptanoic (enanthic); octanoic (caprylic); nonanoic (pelargonic); decanoic (capric); undecanoic (undecylic); dodecanoic (lauric); tridecanoic (tridecylic); tetradecanoic (myristic); pentadecanoic (pentadecylic); hexadecanoic (palmitic); heptadecanoic (margaric); octadecanoic (stearic); nonadecanoic (nonadecylic); eicosanoic (arachidic); heneicosanoic; docosanoic (behenic); tricosanoic; tetracosanoic; pentacosanoic; hexacosanoic (cerotic); heptacosanoic; octacosanoic (montanic); nonacosanoic; triacontanoic (melissic); and the like. Preferred saturated acyl groups are derived from fatty acids selected from the group consisting of: acetic, hexanoic, octanoic, decanoic, hexadecanoic, octadecanoic, and eicosanoic. Most preferred saturated fatty acids are selected from the group consisting of: acetic, hexanoic, octanoic and octadecanoic.

Representative unsaturated free fatty acids (fatty acids) from which the acyl groups may be derived from include, but are not limited to:

1. 10-undecenoic (10-undecylenic);
2. cis-9-tetradecenoic (myristoleic);
3. cis-9-hexadecenoic (palmitoleic);
4. trans-9-hexadecenoic (palmit laidic);
5. cis-6-octadecenoic (petroselinic);
6. trans-6-octadecenoic (petroselaidic);

7. cis-9-octadecenoic (oleic);
8. trans-9-octadecenoic (elaidic);
9. cis-11-octadecenoic (cis-vaccenic);
10. trans-11-octadecenoic (trans-vaccenic);
11. cis-12-hydroxy-9-octadecenoic (ricinoleic);
12. trans-12-hydroxy-9-octadecenoic (ricinelaidic);
13. cis-9,12-octadecadienoic (linoleic);
14. trans-9,12-octadecadienoic (linolelaidic);
15. cis-6,9,12-octadecatrienoic (g-linolenic);
16. cis-9,12,15-octadecatrienoic (linolenic);
17. cis-6,9,12,15-octadecatetraenoic;
18. cis-11-eicosenoic (gondoic);
19. cis-13-eicosenoic;
20. cis-11,14-eicosadienoic;
21. cis-8,11,14-eicosatrienoic;
22. cis-11,14,17-eicosatrienoic;
23. cis-5,8,11,14-eicosatetraenoic (arachidonic);
24. cis-5,8,11,14,17-eicosapentaenoic;
25. cis-13-docosenic (erucic);
26. trans-13-docosenoic (brassidic);
27. cis-13,16-docosadienoic;
28. cis-13,16,19-docosatrienoic;
29. cis-7,10,13,16-docosatrienoic;
30. cis-4,7,10,13,16,19-docosahexanoic;
31. cis-15-tetracosenoic (nervonic); and the like.

Preferred unsaturated fatty acids are selected from the group consisting of: cis-9-octadecenoic; and cis-5,8,11,14-eicosatetraenoic.

Representative diacylglycerols include, but are not limited to:

1. diarachidin (dieicosanoyl-glycerol, reported to be approximately 50% 1,3- and 50% 1,2-isomer);
2. 1,3-diarachidin (1,3-dieicosanoylglycerol);

3. dicaprin (didecanoylglycerol, reported to be 50% 1,3- and 50% 1,2-isomer);

4. 1,3-dicaprin (1,3-didecanoylglycerol);

5. dicaproin (dihexanoylglycerol, reported to be 50% 1,3- and 50% 1,2-isomers);

6. dicaprylin (1,3-dioctanoylglycerol);

7. 1,2-didecanoyl-rac-glycerol (1,2-dicaprin);

8. 1,3-di-cis-11-eicosenoin;

9. 1,3-dielaidin (1,3-di-[(trans)-9-octadecenoyl]glycerol);

10. 1,3-dierucin (1,3-di-[(cis)-13-docosenoyl]-rac-glycerol);

11. 1,2-dihexanoyl-sn-glycerol;

12. dilaurin (didodecanoylglycerol, reported to be approximately 50% 1,3- and 50% 1,2-isomer);

13. 1,3-dilaurin (1,3-didodecanoylglycerol);

14. 1,2-dilauroyl-rac-glycerol (1,2-didodecanoyl-rac-glycerol);

15. dilinolein (1,3-di-[(cis,cis)-9,12-octadecadienoyl]-rac-glycerol);

16. dilinolenin (di-[(cis,cis,cis)-9,12,15-octadecatrienoyl]glycerol);

17. dimyristin (ditetradecanoylglycerol, reported to be approximately 50% 1,3- and 50% 1,2-isomer);

18. 1,3-dimyristin (1,3-ditetradecanoylglycerol);

19. 1,2-dimyristoyl-rac-glycerol (1,2-ditetradecanoyl-rac-glycerol);

20. 1,2-dioctanoyl-rac-glycerol (1,2-dicapryloyl-rac-glycerol);

21. 1,2-dioctanoyl-sn-glycerol (1,2-dicapryloyl-sn-glycerol);

22. diolein (di-[(cis)-9-octadecenoyl]glycerol, reported to be approximately 85% 1,3- and 15% 1,2-isomer);

23. 1,3-diolein (1,3-di-[(cis)-9-octadecenoyl);

24. 1,2-dioleoyl-rac-glycerol (1,2-di[(cis)-9-octadecenoyl]-rac-glycerol);

25. 1,2-dioleoyl-sn-glycerol (1,2-di[(cis)-9-octadecenoyl]-sn-glycerol);

26. dipalmitin (dihexadecanoylglycerol, reported to be approximately 50% 1,2- and 50% 1,3-isomer);

27. 1,3-dipalmitin (1,3-dihexadecanoylglycerol);

28. 1,3-dipalmitolein (1,3-di-[(cis)-9-hexadecenoyl]glycerol);

29. 1,2-dipalmitoyl-sn-glycerol (1,2-dihexadecanoyl-sn-glycerol);

30. 1,2-dipalmitoyl-rac-glycerol (1,2-dihexadecanoyl-rac-glycerol);

31. 1,3-dipentadecanoin (1,3-dipentadecanoylglycerol);

32. distearin (dioctadecanoylglycerol, reported to be approximately 50% 1,3- and 50% 1,2-isomer);

33. 1,3-distearin (1,3-dioctadecanoylglycerol);

34. 1,2-distearoyl-rac-glycerol (1,2-dioctadecanoyl-rac-glycerol);

35. 1-oleoyl-2-acetyl-rac-glycerol (1-[(cis)-9-octadecenoyl]-2-acetyl-rac-glycerol);

36. 1-oleoyl-2-acetyl-sn-glycerol (1-[(cis)-9-octadecenoyl]-2-acetyl-sn-glycerol);

37. 1-palmitoyl-3-stearoyl-rac-glycerol (1-hexadecanoyl-3-octadecanoyl-rac-glycerol);

38. 1-stearoyl-2-arachidonoyl-sn-glycerol (1-octadecanoyl-2-[(cis,cis,cis,cis)-5,8,11,14-eicosatetraenoyl]-sn-glycerol);

39. 1-acetyl-2-oleoylglycerol (1-ethanoyl-2-[(cis)-9-octadecenoylglycerol);

40. 1-stearoyl-2-oleoylglycerol (1-octadecanoyl-2-[(cis)-9-octad cenoylglycerol; and the like.

Preferably the diacylglycerol is selected from the group consisting of:

1. 1,2-dihexanoyl-sn-glycerol;
2. 1,2-dioctanoyl-rac-glycerol;
3. 1,2-dioctanoyl-sn-glycerol;
4. 1-oleoyl-2-acetyl-rac-glycerol;
5. 1-oleoyl-2-acetyl-sn-glycerol;
6. 1-stearoyl-2-arachidonoyl-sn-glycerol;
7. 1,2-didecanoyl-rac-glycerol;
8. 1-acetyl-2-oleoyl glycerol;
9. 1-stearoyl-2-oleoyl glycerol;
10. 1,2-dipalmitoyl-rac-glycerol;
11. 1,2-dipalmitoyl-sn-glycerol;
12. 1,2-distearoyl-rac-glycerol;
13. 1,2-dioleoyl-rac-glycerol;
14. 1,2-dioleoyl-sn-glycerol;
15. diarachidin;
16. 1,3-diarachidin;
17. diolein;
18. 1,3-diolein;
19. dipalmitin;
20. 1,3-dipalmitin;
21. distearin; and
19. 1,3-distearin.

Most preferably the diacylglycerol is selected from the group consisting of: 1,2-dihexanoyl-sn-glycerol; 1,2-dioctanoyl-rac-glycerol; 1,2-dioctanoyl-sn-glycerol; 1-oleoyl-2-acetyl-rac-glycerol; 1-oleoyl-2-acetyl-sn-glycerol, or 1-stearoyl-2-arachidonoyl-sn-glycerol. Most preferably 1,2-dioctanoyl-rac-glycerol or 1,2-dioctanoyl-sn-glycerol is used.

Diacylglycerols are available commercially from, for example: (1) Sigma Chemical Company, St. Louis, MO. --see Sigma's 1989 catalogue of Biochemicals Organic Compounds for Research and Diagnostic Reagents; (2) Serdary Research

Laboratories, Port Huron, MI; (3) Molecular Probes Inc., Junction City, OR; and (4) Avanti Polar Lipids, Birmingham, AL.

Diacylgycerols may also be prepared in accordance with procedures well known in the art, for example see: (1) Gunstone et al., editors, The Lipid Handbook pp. 295, et seq., ©1986; (2) Ebeling et al., Proc. Natl. Acad. Sci. USA, Vol. 82, pp 815-819, at page 816, February 1985; and (3) Ganong et al., Proc. Natl. Acad. Sci. USA, Vol. 83, pp. 1184-1188, March 1986.

Representative triacylglycerols may include but are not limited to:

1. 1,2-dilauroyl-3-myristoyl-rac-glycerol (1,2-didodencanoyl-3-tetradecanoyl-rac-glycerol);
2. 1,2-dimyristoyl-3-lauroyl-rac-glycerol (1,2-ditetradecanoyl-3-dodecanoyl-rac-glycerol);
3. 1,2-dimyristoyl-3-oleoyl-rac-glycerol (1,2-ditetradecanoyl-3-[(cis)-9-octadecenoyl]-rac-glycerol);
4. 1,2-dimyristoyl-3-palmitoyl-rac-glycerol (ditetradecanoyl-3-hexadecanoyl-rac-glycerol);
5. 1,2-dioleoyl-3-palmitoyl-rac-glycerol (1,2-di-[(cis)-9-octadecenoyl]-3-hexadecanoyl-rac-glycerol);
6. 1,3-dioleoyl-2-palmitoylglycerol (1,3-di-[(cis)-9-octadecenoyl]-2-hexadecanoylglycerol);
7. 1,2-dioleoyl-3-(pyren-1-yl)decanoyl-rac-glycerol);
8. 1,2-dioleoyl-3-stearoyl-rac-glycerol (1,2-di-[(cis)-9-octadecenoyl]-3-octadecanoyl-rac-glycerol);
9. 1,3-dioleoyl-2-stearoylglycerol (1,3-di-[(cis)-9-octadecenoyl]-2-octadecanoylglycerol;
10. 1,2-dipalmitoyl-3-myristoyl-rac-glycerol (1,2-dihexadecanoyl-3-tetradecanoyl-rac-glycerol);
11. 1,2-dipalmitoyl-3-oleoyl-rac-glycerol (1,2-dihexadecanoyl-3-[(cis)-9-octadecenoyl]-rac-glycerol);
12. 1,3-dipalmitoyl-2-oleoylglycerol (1,3-dihexadecanoyl-2[(cis)-9-octadecenoyl]glycerol);

13. 1,2-distearoyl-3-myristoyl-rac-glycerol (1,2-dioctadecanoyl-3-tetradecanoyl-rac-glycerol);

14. 1,2-distearoyl-3-oleoyl-rac-glycerol (1,2-dioctadecanoyl-3-[(cis)-9-octadecenoyl]-rac-glycerol);

15. 1,3-distearoyl-2-oleoylglycerol (1,3-octadecanoyl-2-[(cis)-9-octadecanoyl]glycerol);

16. 1,2-distearoyl-3-palmitoyl-rac-glycerol (1,2-dioctadecanoyl-3-hexadecanoyl-rac-glycerol);

17. 1-palmitoyl-2-oleoyl-3-stearoyl-rac-glycerol (1-hexadecanoyl-2-[(cis)-9-octadecenoyl]-3-octadecanoyl-rac-glycerol);

18. triacetin (1,2,3-triacetylglycerol; glyceryl triacetate);

19. triarachidin (1,2,3-trieicosanoylglycerol);

20. triarachidonin (1,2,3-tri-[(cis,cis,cis,cis)-5,8,11,14-eicosatetraenoyl]glycerol);

21. tribehenin (1,2,3-tridocosanoylglycerol);

22. tributyrin (1,2,3-tributyrylglycerol; glyceryl tributyrate);

23. tricaprin (1,2,3-tridecanoylglycerol);

24. tricaproin (1,2,3-trihexanoylglycerol; trihexanoin);

25. tricaprylin (1,2,3-trioctanoylglycerol; glyceryl tricaprylate);

26. tri-11-eicosenoin (1,2,3-tri-[(cis)-11-eicosenoyl]-glycerol);

27. trielaidin (1,2,3-tri-[(trans)-9-octadecenoyl]glycerol);

28. trierucin (1,2,3-tri-[(cis)-13-docosenoyl]glycerol);

29. triheptadecanoin (1,2,3-triheptadecanoylglycerol);

30. trilaurin (1,2,3-tridodecanoylglycerol);

31. trilinolelaidin (1,2,3-tri-[(trans,trans)-9,12-octadecadienoyl]glycerol);

32. trilinolein (1,2,3-tri-[(cis,cis)-9,12-octadecadienoyl]glycerol);

33. trilinolenin (1,2,3-tri-[(cis,cis,cis)-9,12,15-octadecatrienoyl]-glycerol);

34. trimyristin (1,2,3-tritetradecanoylglycerol);

35. trimyristolein (1,2,3-tri-[(cis)-9-tetradecenoyl]-glycerol);

36. trinervonin (1,2,3-tri-[(cis)-15-tetracosenoyl]glycerol);

37. trinonadecanoin (1,2,3-trinonadecanoylglycerol);

38. trinonanoin (1,2,3-trinonanoylglycerol; pelargonin);

39. triolein (1,2,3-tri-[(cis)-9-octadecenoyl]glycerol; glyceryl trioleate);

40. tripalmitin (1,2,3-trihexadecanoylglycerol);

41. tripalmitolein (1,2,3-tri-[(cis)-9-hexadecenoyl]-glycerol);

42. tripentadecanoin (1,2,3-tripentadecanoylglycerol);

43. tripetroselinin (1,2,3-tri-[(cis)-6-octadecenoyl]-glycerol);

44. tristearin (1,2,3-trioctadecanoylglycerol);

45. tritridecanoin (1,2,3-tritridecanoylglycerol);

and the like. These triacylglycerols are commercially available from Sigma Chemical Company (same address and catalogue as cited above). Triacylglycerols may also be prepared in accordance with procedures well known in the art, for example see Gunstone et al., editors, The Lipid Handbook, p. 295 et seq., ©1986.

Lipopolysaccharides (LPS) may also be useful in this invention as PK-C Activators. The active lipid moiety of LPS of Gram-negative bacteria is diacylglucosamine 1-phosphate. Thus, either a diacylglucosamine 1-phosphate or the LPS containing it

may be used. The acyl groups of the diacylglucosamine 1-phosphates from LPS are usually from predominantly C_{14} to C_{18} fatty acids which may be saturated or monosaturated, but not polyunsaturated. On LPS and bacterial fatty acids see, for example, Davis et al., editors, Microbiology, Third Edition, pp 82 to 91, ©1980, the disclosure of which is incorporated herein by reference thereto.

Representative examples of bacteria from which LPS can be derived from for use in this invention include, but are not limited to: Escherichia coli (E. coli), Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella abortus equi, Salmonella enteritidis, Salmonella minnesota, Salmonella typhimurium, Salmonella typhosa, Serratia marcescens, Shigella flexneri, Vibrio cholerae, and the like. Bacterial Lipid A and Lipid X may also prove useful. Lipid A and Lipid X are well known to those skilled in the art. See for example: (1) Wightman et al., The Journal of Biological Chemistry, Vol. 259, No. 16, pp 10048-10052, August 25, 1984; and (2) Davis et al., editors, Microbiology, Third Edition, pp. 85, 87, and 654-655, ©1980; the disclosures of each being incorporated herein by reference thereto. Lipid A is commercially available from, for example, Sigma Chemical Company. Lipid X is available from Lipidex, Inc., Middleton, WI.

LPS are available commercially, for example, form Sigma Chemical Co. (already cited above). Examples of LPS available comercially include those derived from: E. coli Serotype 026:B6; E. coli Serotype 055:B5; E. coli Serotype 0111:B4; E. coli Serotype 0127:B8; E. coli Serotype 0128:B12; E. coli EH-100 (Ra mutant); E. coli F-583 (Rd mutant); E. coli Strain J5 (Rc mutant); E. coli K235, Klebsiella pneumonia;, Pseudomonas aeruginosa; Pseudomonas aeruginosa Serotype 10 (Habs); Salmonella abortus equi, Salmonella enteritidis, Salmonella Minnesota, Salmon lla Minnesota Strain R5; Salmonella Minnesota Strain R7 (Rd mutant); Salmonella Minnesota Strain Re 595 (Re mutant); Salmonella typhimurium; Salmonella

typhimurium Strain TV119 (Ra mutant); Salmonella typhimurium Strain SL684 (Rc mutant); Salmonella typhimurium Strain SL1181 (Re mutant); Salmonella typhosa, Serratia marcescens; Shigella flexneri Serotype 1A; Shigella flexneri (Re mutant); and Vibrio cholerae Serotype INABA 569B.

LPS may be derived from bacteria by techniques well known to those skilled in the art. For example, lyophilized powders are available as phenol, trichloroacetic acid (TCA), butanol or phenol-chloroform-petroleum ether extracts. Such procedures are referenced in Sigma Chemical Company's 1989 Biochemicals Organic Compounds catalogue (cited above) as: Westphal et al., Methods in Carbohydrate Chem., 5, 83 (1965) for a phenol extraction procedure; Staub, Methods in Carbohydrate Chem., 5, 92 (1965) for a TCA extraction procedure; Lieve et al., Methods in Enzymology, XXVIIIb, 254 (1972) for a butanol extraction procedure; and Galanos et al., Eur. J. Biochem., 9, 245 (1969) for a phenol-chloroform-petroleum ether extraction.

Unsaturated free fatty acids (fatty acids) may also be useful in this invention as PK-C Activators. It is believed that unsaturated free fatty acids having 1 to about 4 double bonds and about 14 to about 20 carbon atoms are preferred PK-C Activators. Cis- and trans-unsaturated free fatty acids are suitable with the proviso that trans-elaidic acid may not be as useful as other unsaturated free fatty acids. Although chain lengths of 14-20 carbon atoms are preferred other chain lengths (less than 14 or more than 20) may also prove useful. Suitable unsaturated free fatty acids may be selected from amongst those unsaturated fatty acids already described above for the acyl groups of the diacylglycerols and triacylglycerols. Preferred unsaturated free fatty acids include linoleic acid, arachidonic acid and oleic acid.

Short chain saturated free fatty acids (fatty acids) may also prove useful. Suitable saturated free fatty acids may be selected from amongst those saturated fatty acids, having 4 to 10 carbon atoms, described above for the acyl groups of the

diacylglycerols and the triacylglycerols. Saturated fatty acids having more than 10 carbon atoms --e.g., 11-20-- may also prove useful. Thus, lauric, myristic, palmitic, stearic, and arachidic may be suitable.

Another group of compounds which may be useful in this invention as PK-C Activators for enhancing melanin production are glycerophospholipids (phosphoglycerides). Phosphoglycerides consist of a glycerol background, two acyl groups derived from fatty acids (usually bound to the C-1 and C-2 glycerol carbons) and a phosphorylated alcohol. The major phosphoglycerides are derivatives of phosphatidate (diacylglycerol 3-phosphate). The phosphate group of phosphatidate becomes esterified to the hydroxyl group of one of several alcohols. Examples of alcohols include serine, threonine, ethanolamine, choline, glycerol, inositol, and the like. The disclosure above pertaining to the acyl groups of the di- and triacylglycerols pertain equally as well to the acyl groups of the phosphoglycerides.

Representative examples of phosphoglycerides include, but are not limited to:

1. L-α-phosphatidylcholine (L-α-lecithin) such as that obtained from bovine brain, bovine heart, bovine liver, egg yolk (diced, fresh, frozen or fresh frozen), turkey egg yolk (fresh), and soybean;

2. L-α-phosphatidylcholine, β-acetyl-γ-O-alkyl (1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine);

3. D-α-phosphatidylcholine, β-acetyl-γ-O-hexadecyl;

4. DL-α-phosphatidylcholine, β-acetyl-γ-O-hexadecyl;

5. L-α-phosphatidylcholine, β-acetyl-γ- O-hexadecyl;

6. L-α-phosphatidylcholine, β-acetyl-γ- O-(octadec-9-cis-enyl);

7. L-α-phosphatidylcholine, β-O-acetyl-γ-O-octadecyl;

8. L-α-phosphatidylcholine, β-acetyl-γ-oleoyl (1-[(cis)-9-octadecenoyl]-2-acetyl-sn-glycero-3-phosphocholine);

9. L-α-phosphatidylcholine, β-arachidonoyl, γ-stearoyl (1-octadecanoyl-2-[(cis,cis,cis,cis)-5,8,11,14-eicosatetraenoyl]-sn-glycero-3-phosphocholine);

10. L-α-phosphatidylcholine, diarachidoyl;

11. L-α-phosphatidylcholine, dibehenoyl;

12. L-α-phosphatidylcholine, dibutyroyl;

13. L-α-phosphatidylcholine, dicaproyl;

14. L-α-phosphatidylcholine, didecanoyl;

15. L-α-phosphatidylcholine, dielaidoyl;

16. L-α-phosphatidylcholine, diheptadecanoyl;

17. L-α-phosphatidylcholine, diheptanoyl;

18. DL-α-phosphatidylcholine, di-O-hexadecyl (1,2-di-O-hexadecyl-rac-glycero-3-phosphocholine);

19. DL-α-phosphatidylcholine, dilauroyl (1,2-didodecanoyl-rac-glycero-3-phosphocholine);

20. L-α-phosphatidylcholine, dilauroyl;

21. L-α-phosphatidylcholine, dilinoleoyl;

22. L-α-phosphatidycholine, dimyristoyl;

23. L-α-phosphatidylcholine, dinonanoyl (1,2-dinonanoyl-sn-glycero-3-phosphocholine);

24. L-α-phosphatidylcholine, dioctanoyl (1,2-dioctanoyl-sn-glycero-3-phosphocholine);

25. DL-α-phosphatidylcholine, dioleoyl;

26. L-α-phosphatidylcholine, dioleoyl;

27. D-α-phosphatidylcholine, dipalmitoyl (2,3-dihexadecanoyl-sn-glycero-1-phosphocholine);

28. DL-α-phosphatidylcholine, dipalmitoyl;

29. L-α-phosphatidylcholine, dipalmitoyl;

30. L-α-phosphatidylcholine, dipentadecanoyl (1,2-dipentadecanoyl-sn-glycero-3-phosphocholine);

31. L-α-phosphatidylcholine, distearoyl;

32. L-α-phosphatidylcholine, diundecanoyl (1,2-diundecanoyl-sn-glycero-3-phosphocholine);

33. L-α-phosphatidylcholine, divaleroyl;

34. L-α-phosphatidylcholine, β-elaidoyl-γ-palmitoyl;

35. L-α-phosphatidylcholine, β-linoleoyl-γ-palmitoyl;

36. DL-α-phosphatidylcholine, β-O-methyl-γ-O-hexadecyl (1-O-Hexadecyl-2-O-methyl-rac-glycero-3-phosphocholine);

37. L-α-phosphatidylcholine, β-O-methyl-γ-O-octadecyl;

38. L-α-phosphatidylcholine, β-(NBD-aminohexanoyl)-γ-palmitoyl (1-hexadecanoyl-1-[(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-aminohexanoyl]-sn-glycero-3-phosphocholine);

39. DL-α-phosphatidylcholine, β-oleoyl-γ-O-hexadecyl (1-O-hexadecyl-2-[(cis)-9-octadecenoyl]-rac-glycero-3-phosphocholine);

40. L-α-phosphatidylcholine, β-oleoyl-γ-palmitoyl;

41. L-α-phosphatidylcholine, β-oleoyl-γ-stearoyl;

42. DL-α-phosphatidylcholine, β-palmitoyl-γ-O-hexadecyl (1-O-hexadecyl-2-hexadecanoyl-rac-glycero-3-phosphocholine);

43. L-α-phosphatidylcholine, β-palmitoyl-γ-oleoyl;

44. L-α-phosphatidylcholine, β-palmitoyl-γ-(pyren-1-yl)-hexanoyl;

45. L-α-phosphatidylcholine, β-(pyren-1-yl)decanoyl-γ-palmitoyl;

46. L-α-phosphatidylcholine, β-(pyren-1-yl)hexanoyl-γ-palmitoyl;

47. L-α-phosphatidylcholine, β-stearoyl-γ-oleoyl;

48. DL-α-phosphatidyl-N,N-dimethylethanolamine, dipalmitoyl;

49. L-α-phosphatidyl-N,N-dimethylethanolamine, dipalmitoyl;

50. L-α-phosphatidylethanolamine (L-α-cephalin), such as that obtained from bovine brain, sheep brain, egg yolk, soybean, Escherichia coli, dog brain, bovine liver, or porcine liver;

51. L-α-phosphatidylethanolamine, diheptadecanoyl (1,2-diheptadecanoyl-sn-glycero-3-phosphoethanolamine);

52. L-α-phosphatidylethanolamine, dilauroyl (1,2-didodecanoyl-sn-glycero-3-phosphoethanolamine);

53. L-α-phosphatidylethanolamine, dimyristoyl (1,2-ditetradecanoyl-sn-glycero-3-phosphoethanolamine);

54. phosphatidylethanolamine, dinitrophenyl;

55. L-α-phosphatidylethanolamine, dioleoyl (1,2-di[(cis)-9-octa-decenoyl]-sn-glycero-3-phosphoethanolamine);

56. DL-α-phosphatidylethanolamine, dipalmitoyl;

57. L-α-phosphatidylethanolamine, dipalmitoyl;

58. L-α-phosphatidylethanolamine, dipalmitoyl-N-dansyl (1,2-dihexadecanoyl-sn-glycero-3-phospho-[N-dansyl]ethanolamine);

59. L-α-phosphatidylethanolamine, dipalmitoyl, N-fluorescein isothiocyanyl (1,2-dihexadecanoyl-sn-glycero-3-phospho-[N-fluorescein isothiocyanyl]ethanolamine) sodium salt;

60. L-α-phosphatidylethanolamine, dipalmitoyl, N-NBD (1,2-dihexadecanoyl-sn-glycero-3-phospho-[N-(4-nitrobenzo-2-oxa-1,3-diazole)]ethanolamine);

61. L-α-phosphatidylethanolamine, distearoyl (1,2-dioctadecanoyl-sn-glycero-3-phosphoethanolamine);

62. L-α-phosphatidylethanolamine, β-linoleoyl-γ-palmitoyl (1-hexadecanoyl-2-[(cis,cis)-9,12,octadecadienoyl]-sn-glycero-3-phosphoethanolamine);

63. L-α-phosphatidylethanolamine, β-oleoyl-γ-palmitoyl;

64. phosphatidylethanolamine, plasmalogen;

65. phosphatidylethanolamine, N-trinitrophenyl;

66. L-α-phosphatidyl-DL-glycerol (1-[3-sn-phosphatidyl]-rac-glycerol) [prepared by reaction of cabbage phospholipase D with egg yolk L-α-phosphatidylcholine in the presence of glycerol], including the ammonium salt from egg yolk lecithin and the sodium salt from egg yolk lecithin;

67. L-α-phosphatidyl-DL-glycerol, dimyristoyl (1,2-ditetradecanoyl-sn-glycero-3-[phospho-rac-(1-glycerol)]), including the ammonium and sodium salts;

68. L-α-phosphatidyl-DL-glycerol, dioleoyl (1,2-di[(cis)-9-octadecenoyl]-sn-glycero-3-[phospho-rac-(1-glycerol)]), ammonium salt;

69. DL-α-phosphatidyl-DL-glycerol, dipalmitoyl (1,2-dipalmitoyl-rac-glycero-3-[phospho-rac-(1-glycerol)]), including the ammonium salt;

70. L-α-phosphatidyl-DL-glycerol, dipalmitoyl (1,2-dihexadecanoyl-sn-glycero-3-[phospho-rac-(1-glycerol)]), including the ammonium and sodium salts;

71. L-α-phosphatidyl-DL-glycerol, distearoyl (1,2-distearoyl-sn-glycero-3-[phospho-rac-(1-glycerol)]) ammonium salt;

72. L-α-phosphatidylinositol, e.g. from soybean (including the ammonium and sodium salts), and from bovine liver (ammonium salt), as well as TYPE 1: Folch Fraction 1 from bovine brain reported to contain 10-20% phosphatidyl inositides, 50-60% phosphatidyl serine as well as several other brain lipids;

73. L-α-phosphatidylinositol 4,5-diphosphate (triphosphoinositide) sodium salt from bovine brain;

74. L-α-phosphatidylinositol 4-monophosphate (diphosphoinositide) sodium salt from bovine brain;

75. phosphoinositides, sodium salt, from bovine brain, Extract Type 1, reported to contain approximately 15-20% phosphatidylinositol 4-monophosphate and phosphatidylinositol 4,5-biphosphate with the remainder being a mixture of phosphatidylinositol and phosphatidylserine;

76. L-α-phosphatidyl-N-monomethylethanolamine, dipalmitoyl;

77. L-α-phosphatidyl(N-palmitoyl)ethanolamine, dipalmitoyl (1,2-dihexadecanoyl-sn-glycero-3-phospho-[N-hexadecanoyl]ethanolamine) ammonium salt;

78. L-α-phosphatidyl-L-serine, e.g., from bovine brain (including the sodium salt), as well as TYPE III: Folch Fraction III from bovine brain reported to contain 80-85% phosphatidylserine with the balance being other brain lipids;

79. L-α-phosphatidylserine, dansyl; and

80. DL-α-phosphatidyl-L-serine, dipalmitoyl.

In general the PK-C Activator is in a concentration which is effective to provide the desired level of activity. The PK-C Activator may be used in amounts of about 0.01% to about 20% by weight of the total composition with about 0.05% to about 10% being preferred and about 0.05% to about 1.0% being most preferred. Combinations of PK-C Activators may be used such that their total amount is within the specified ranges.

Suitable solvents for use with the PK-C Activators include liposomes; ketones such as acetone and the like; alcohols such as benzyl alcohol, ethanol, t-butyl alcohol, cetyl alcohol, glycol ($HOCH_2CH_2OH$), isopropyl alcohol, propylene glycol, SD alcohol 23-A, SD alcohol 39-C, SD alcohol 40, SD alcohol 40-B and the like; Fats and oils such as avocado oil, cocoa butter, coconut oil, corn oil, hydrogenated coconut oil, hydrogenated cottonseed oil, hydrogenated vegetable oil, lanolin oil, mink oil, palm oil, peanut oil, safflower oil, soybean oil, sunflower seed oil, sweet almond oil, vegetable oil (expressed oil of vegetable origin consisting primarily of triglycerides of fatty acids), walnut oil, wheat germ oil and the like; hydrocarbons such as mineral oil and the like; alkoxylated alcohols or polymeric ethers such as PEG-8, PEG-14M and the like; lanolin and lanolin derivatives such as hydrogenated lanolin and the like; glyceryl esters and derivatives such as hydrogenated palm kernel oil and the like; esters such as isopropyl myristate, isopropyl palmitate

and the like; water; and the like. Addition solvents which may be used can be found on Nikitakis, Editor, CTFA Cosmetic Ingredient Handbook, First Edition, published by the Cosmetic, Toiletry and Fragrance Association, Inc., 1110 Vermont Avenue, N.W., Washington, D.C., ©1988, the disclosure of which is incorporated herein by reference thereto. In particular, see the section "Solvents" on pp. 85-86. The disclosure above relating to the encapsulation of riboflavin, riboflavin phosphate or mixtures thereof in liposomes applys equally well to the PK-C Activators. The liposomes may also be used as a solvent for the PK-C Activators or may used to blend with the PK-C Activators so that the PK-C Activators are in the lipid layer. The riboflavin, riboflavin phosphate or mixtures thereof may be combined with the PK-C Activators and the resultant combination may be encapsulated in liposomes.

Since, in general, PK-C Activators are lipophilic, it may be desirable to add components to the water when making up aqueous solutions for encapsulation in liposomes. These added components would be water miscible and would improve the water solubility of the PK-C Activators. These components may include solvents such as alcohols and ketones already discussed above.

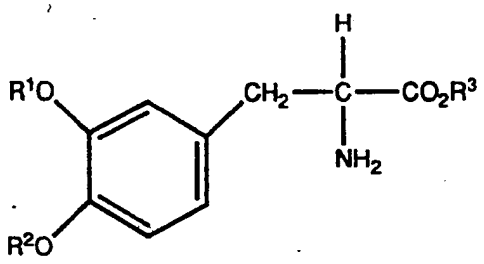
It is desirable to use compositions containing PK-C Activators immediately after they are prepared or to freeze them at about -20°C until they are used. If this is not convenient then it is necessary to add an effective amount of at least one antioxidant to protect the PK-C Activator from degradation. However, if a PK-C Activator is used which will not degrade over time then an antioxidant is no longer necessary but its use is still preferred. Generally, about 0.05 to about 0.10% by weight of the total composition of an antioxidant is sufficient. Any of the antioxidants known for use in the cosmetics industry may be used. Examples of antioxidants include but are not limited to beta-carotene, BHA, BHT, a-tocopherol, propyl gallate, sodium bisulfite, sodium

metabisulfite, ascorbyl dipalmitate, TENOX (trademark for food grade antioxidants reported to contain one or more of the following ingredients: butylated hydroxyanisole, butylated hydroxytoluene, and/or propyl gallate with or without citric acid; some formulas are supplied in solvents such as propylene glycol), and the like. See, for example CTFA Cosmetic Ingredient Handbook cited above.

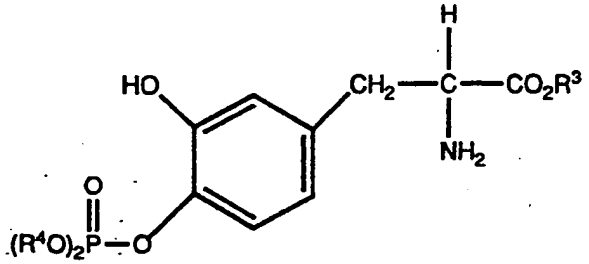
If desirable, at least one antioxidant may be added to the compositions containing riboflavin, riboflavin phosphate or mixtures thereof but no PK-C Activator. The amount of antioxidant used would be the same as discussed above for use with the PK-C Activators.

DOPA phosphates can be used in amounts of about 0.005% to about 1.0% by weight of the total composition with about 0.015% to about 0.5% being preferred and about 0.05% to about 0.02% being most preferred.

The DOPA phosphates (phosphodopas) are O-phosphorylated derivatives of DOPA. The DOPA phosphates are represented by Formulas I-V:



(I)



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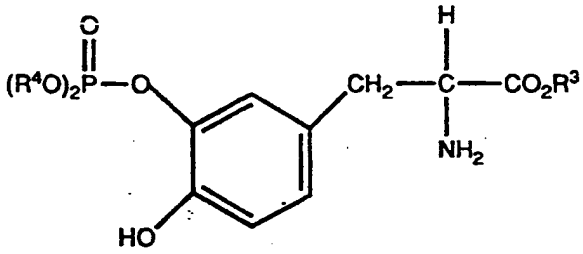

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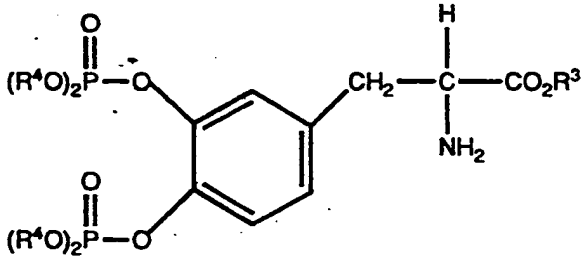

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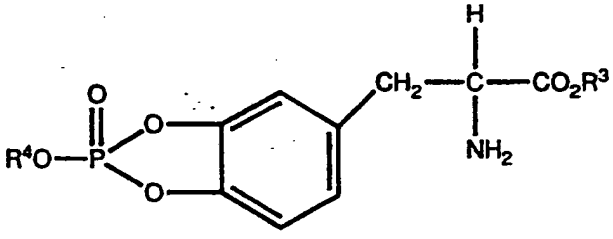
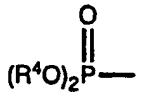
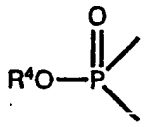


(V)

wherein R^1 and R^2 each represent hydrogen or



R^1 and R^2 together represent



wherein R^4 and R^3 each represent hydrogen or a pharmaceutically acceptable cation; with the proviso that R^1 and R^2 cannot both be hydrogen.

The sunscreening agents used can be of the UVA type, UVB type, or a combination of both. Generally, the sunscreening agents are used in amounts effective to provide the desired level of protection against UVA and/or UVB radiation. Usually, the sunscreening agents are used in amounts of about 2% to about 20% by weight of the total composition with about 5% to about 18% being preferred and about 2% to about 15% being most preferred.

Typical UVB type sunscreening agents include substituted para-aminobenzoates, alkyl esters of para-methoxycinnamate and certain esters of salicylic acid.

Typical UVA type sunscreening agents include certain benzophenones and dibenzoyl methanes.

Representative UVB type sunscreening agents include but are not limited to:

(A) DEA Methoxyinnamate (diethanolamine salt of p-methoxy hydro cinnamate), e.g., tradename BERNEL HYDRO from Bernel Chemical Co., Inc.;

(B) Ethyl Dihydroxypropyl PABA (ethyl dihydroxypropyl p-aminobenzoate), e.g., tradename AMERSCREEN P from Amerchol Corp.;

(C) Glyceryl PABA (glyceryl-p-aminobenzoate), e.g., tradename NIPA G.M.P.A. from NIPA Laboratories, Inc.;

(D) Homosalate (Homomenthyl salicylate), e.g., tradename KEMESTER HMS from Humko Chemical;

(E) Octocrylene (2-ethylhexyl-2-cyano-3,3-diphenylacrylate), e.g., tradename UVINUL N-539 from BASF Chemical Co.;

(F) Octyl Dimethyl PABA (Octyl dimethyl p-aminobenzoate, 2-ethylhexyl p-dimethylaminobenzoate, Padimate O), e.g., tradenames AMERSCOL, ARLATONE UVB, and ESCALOL 507 from Amerchol Corp., ICI Americas, Inc., and Van Dyk, respectively;

(G) Octyl Methoxycinnamate (2-ethylhexyl-p-methoxycinnamate), e.g., tradename PARSOL MCX from Bernel Chemical Co. Inc., or Givaudan Corp.;

(H) Octyl Salicylate (2-ethylhexy salicylate), e.g., tradename SUNAROME WMO from Felton Worldwide, Inc.;

(I) PABA (p-amino benzoic acid), e.g., tradename PABA from EM Industries, Inc. and National

Starch & Chemical Corp., or tradename NIPA PABA from NIPA Laboratories Inc.;

(J) 2-Phenyl-benzimidazole-5-Sulphonic acid (Novantisol), e.g., tradename EUSOLEX 232 and NEO-HELIOPAN HYDRO from EM Industries, Inc. and Haarmann & Reimer Corp., respectively;

(K) TEA Salicylate (triethanolamine salicylate), e.g., tradenames SUNAROME W and SUNAROME G from Felton Worldwide, Inc.;

(L) 3-(4-methylbenzlidene)camphor or 3-(4-methylbenzylidene)boran-2-one, e.g., tradename EUSOLEX 6300 from EM Industries, Inc.; and

(M) Etocrylene (2-ethyl-2-cyano-3,3'-diphenylacrylate), e.g., tradename UVINUL N-35 from BASF Chemical Co.

Representative UVA type sunscreening agents include but are not limited to:

(A) Benzophenone-3 (2-hydroxy-4-methoxy-benzophenone), e.g., tradename SPECTRA-SORB UV-9 and UVINUL M-40 from American Cyanamid Co. and BASF Chemical Co., respectively;

(B) Benzophenone-4 (sulisobenzone), e.g., tradename UVINUL MS-40 from BASF Chemical Co.;

(C) Benzophenone-8 (dioxybenzone), e.g., tradename SPECTRA-SORB UV-24 from American Cyanamid Co.;

(D) Menthyl Anthranilate (Menthyl-O-aminobenzoate), e.g., tradename SUNAROME UVA from Felton Worldwide, Inc.;

(E) Benzophenone-1 (2,4-dihydroxybenzophenone), e.g., tradename UVINUL 400 and UVASORB 2 OH from BASF Chemical Co. and TRI-K Industries, Inc., respectively;

(F) Benzophenone-2 (2,2',4,4'-tetrahydroxy-benzohpenone), e.g., tradename UVINUL D-50 from BASF Chemical Co.;

(G) Benzophenone-6 (2,2'-dihydroxy-4,4'-dimethoxy-benzophenone), e.g., tradename UVINUL D-49 from BASF Chemical Co.;

(H) Benzophenone-12 (octabenzone), e.g., tradename UVINOL 408 from BASF Chemical Co.;

(I) 4-isopropyl dibenzoyl methane (1-p-cumenyl-3-phenylpropane-1,3-dione), e.g. tradename EUSOLEX 8020 from EM Industries, Inc.; and

(J) Butyl methyl dibenzoyl methane (4-t-butyl-4'-methoxydibenzoyl methane), e.g. tradename PARSOL 1789 from Givaudan Corporation;

Physical sunscreening agents may also be used. For example, red petrolatum in amounts of about 30 to about 99% by weight of the total composition, or titanium dioxide in amounts

of about 2 to about 25% by weight of the total composition may be used. Talc, kaolin, chalk , and precipitated silica may also be used in effective amounts, e.g., about 1% to about 10% by weight of the total composition.

Additional sunscreening agents include lawsone (hydroxynaphthoquinone, $C_{10}H_6O_3$, the coloring matter of henna leaves) with dihydroxy acetone.

Usually, when used, at least one UVB type and at least one UVA type sunscreening agent is used.

For example, at least one of the following UVB type sunscreening agents can be used: from about 1.5 to about 8.0% by weight of the total composition of octyl dimethyl PABA; octyl para- methoxycinnamate in amounts of about 1.5 to about 7.5% by weight of the total composition; homomenthyl salicylate in amounts of about 4.0 to about 15% by weight of the total composition; and octyl salicylate in amounts of about 3 to about 5% by weight of the total composition.

Also, for example, at least one of the following UVA type sunscreening agents can be used: benzophenone-3 in amounts of about 0.5 to about 6% by weight of the total composition; benzophenone-8 in amounts of about 0.5 to about 3% by weight of the total composition; and menthyl anthranilate in amounts of about 3.5 to about 5.0% by weight of the total composition.

Using the ingredients disclosed above (e.g., emollients, emulsifiers, film formers, and the like), the riboflavin, riboflavin phosphate or mixtures thereof can be incorporated into formulations such as lotions, creams, gels mousses, waxed based sticks, aerosols, alcohol sticks and the like. These formulations are well known in the art, for example see Balsam, M.S., and Sagrin, E. (Editors) Cosmetic Science and Technology, Second Edition, Volumes 1 and 2, Wiley-Interscience, a division of John Wiley & Sons, Inc., New York, copyright 1972; and Flick E.W., Cosmetic and Toiletry Formulations, Noyes Publications, 1984.

Emollients may be used in amounts which are effective to prevent or relieve dryness. Useful emollients may include: hydrocarbon oils and waxes; silicone oils; triglyceride esters; acetoglyceride esters; ethoxylated glyceride; alkyl esters; alkenyl esters; fatty acids; fatty alcohols; fatty alcohol ethers; ether-esters; lanolin and derivatives; polyhydric alcohols (polyols) and polyether derivatives; polyhydric alcohol (polyol) esters; wax esters; beeswax derivatives; vegetable waxes; phospholipids; sterols; and amides.

Thus, for example, typical emollients include mineral oil, especially mineral oils having a viscosity in the range of 50 to 500 SUS, lanolin oil, mink oil, coconut oil, cocoa butter, olive oil, almond oil, macadamia nut oil, aloe extract, jojoba oil, safflower oil, corn oil, liquid lanolin, cottonseed oil, peanut oil, purcellin oil, perhydrosqualene (squalene), caster oil, polybutene, odorless mineral spirits, sweet almond oil, avocado oil, calophyllum oil, ricin oil, vitamin E acetate, olive oil, mineral spirits, cetearyl alcohol (mixture of fatty alcohols consisting predominantly of cetyl and stearyl alcohols), linolenic alcohol, oleyl alcohol, octyl dodecanol, the oil of cereal germs such as the oil of wheat germ cetearyl octanoate (ester of cetearyl alcohol and 2-ethylhexanoic acid), cetyl palmitate, diisopropyl adipate, isopropyl palmitate, octyl palmitate, isopropyl myristate, butyl myristate, glyceryl stearate, hexadecyl stearate, isocetyl stearate, octyl stearate, octylhydroxy stearate, propylene glycol stearate, butyl stearate, decyl oleate, glyceryl oleate, acetyl glycerides, the octanoates and benzoates of (C_{12}-C_{15}) alcohols, the octanoates and decanoates of alcohols and polyalcohols such as those of glycol and glycerol, and ricin- oleates of alcohols and poly alcohols such as those of isopropyl adipate, hexyl laurate, octyl dodecanoate, dimethicone copolyol, dimethiconol, lanolin, lanolin alcohol, lan lin wax, hydrogenated lanolin, hydroxylated lanolin, acetylated lanolin, petrolatum, isopropyl lanolate, cetyl myristate, glyceryl myristate, myristyl myristate, myristyl

lactate, cetyl alcohol, isostearyl alcohol stearyl alcohol, and isocetyl lanolate, and the like.

Emulsifiers (emulsifying agents) may be used in amounts effective to provide uniform blending of ingredients of the composition. Useful emulsifiers may include

A. Anionics

1. Fatty acid soaps, e.g., potassium stearate, sodium stearate, ammonium stearate, and triethanolamine stearate;

2. Polyol fatty acid monoesters containing fatty acid soaps, e.g., glycerol monostearate containing either potassium or sodium salt;

3. Sulfuric esters (sodium salts), e.g., sodium lauryl sulfate, and sodium cetyl sulfate; and

4. Polyol fatty acid monoesters containing sulfuric esters, e.g., glyceryl monostearate containing sodium lauryl sulfate;

B. Cationics

1. N(stearoyl colamino formylmethyl) pyridium chloride;

2. N-soya-N-ethyl morpholinium ethosulfate;

3. Alkyl dimethyl benzyl ammonium chloride;

4. diisobutylphenoxytheoxyethyl dimethyl benzyl ammonium chloride; and

5. cetyl pyridium chloride;

C. Nonionics

1. polyoxyethylene fatty alcohol ethers, e.g., polyoxyethylene lauryl alcohol;

2. polyoxypropylene fatty alcohol ethers, e.g., propoxylated oleyl alcohol;

3. polyoxyethylene fatty acid esters, e.g., polyoxyethylene stearate;

4. polyoxyethylene sorbitan fatty acid esters, e.g., polyoxyethylene sorbitan monostearate;

5. sorbitan fatty acid esters, e.g., sorbitan monostearate;

6. polyoxyethylene glycol fatty acid esters, e.g., polyoxyethylene glycol monostearate;

7. polyol fatty acid esters, e.g., glyceryl monostearate and propylene glycol monostearate; and

8. ethoxylated lanolin derivatives, e.g., ethoxylated lanolins, ethoxylated lanolin alcohols and ethoxylated cholesterol.

Surfactants may also be used in the compositions of this invention. Suitable surfactants may include those generally grouped as cleansing agents, emulsifying agents, foam boosters, hydrotropes, solubilizing agents, suspending agents and nonsurfactants (facilitates the dispersion of solids in liquids). The surfactants are usually classified as amphoteric, anionic, cationic and nonionic surfactants.

Amphoteric surfactants include acylamino acids and derivatives and N-alkylamino acids.

Anionic surfactants include: acylamino acids and salts, such as, acylglutamates, acylpeptides, acylsarcosinates, and acyltaurates; carboxylic acids and salts, such as, alkanoic acids, ester carboxylic acids, and ether carboxylic acids; sulfonic acids and salts, such as, acyl isethionates, alkylaryl sulfonates, alkyl sulfonates, and sulfosuccinates; sulfuric acid esters, such as, alkyl ether sulfates and alkyl sulfates.

Cationic surfactants include: alkylamines, alkyl imidazolines, ethoxylated amines, and quaternaries (such as, alkylbenzyldimethylammonium salts, alkyl betaines, heterocyclic ammonium salts, and tetra alkylammonium salts).

Nonionic surfactants include: alcohols, such as primary alcohols containing 8 to 18 carbon atoms; alkanolamides such as alkanolamine derived amides and ethoxylated amides; amine oxides; esters such as ethoxylated carboxylic acids, ethoxylated glycerides, glycol esters and derivatives, monoglycerides, polyglyceryl esters, polyhydric alcohol esters

and ethers, sorbitan/sorbitol esters, and triesters of phosphoric acid; and ethers such as ethoxylated alcohols, ethoxylated lanolin, ethoxylated polysiloxanes, and propoxylated polyoxyethylene ethers.

Useful solvents for sunscreening agents include those solvents already disclosed as being useful solvents for the PK-C Activators.

Suitable waxes which may prove useful include: animal waxes, such as beeswax, spermaceti, or wool wax (lanolin); plant waxes, such as carnauba or candelilla; mineral waxes, such as montan wax or ozokerite; and petroleum waxes, such as paraffin wax and microcrystalline wax (a high molecular weight petroleum wax). Animal, plant, and some mineral waxes are primarily esters of a high molecular weight fatty alcohol with a high molecular weight fatty acid. For example, the hexadecanoic acid ester of tricontanol is commonly reported to be a major component of beeswax.

Suitable waxes which may be useful also include the synthetic waxes including polyethylene polyoxyethylene and hydrocarbon waxes derived from carbon monoxide and hydrogen (Fischer-Tropsch synthesis).

Representative waxes also include: ceresin; cetyl esters; hydrogenated jojoba oil; hydrogenated jojoba wax; hydrogenated rice bran wax; Japan wax; jojoba butter; jojoba oil; jojoba wax; munk wax; montan acid wax; ouricury wax; rice bran wax; shellac wax; sufurized jojoba oil; synthetic beeswax; synthetic jojoba oils; trihydroxystearin; cetyl alcohol; stearyl alcohol; cocoa butter; fatty acids of lanolin; mono-, di- and triglycerides which are solid at 25°C, e.g., glyceyl tribehenate (a triester of behenic acid and glycerine) and C_{18}-C_{36} acid triglyceride (a mixture of triesters of C_{18}-C_{36} carboxylic acids and glycerine) available from Croda, Inc., New York, NY under the tradenames Syncrowax HRC and Syncrowax HGL-C, respectively; fatty sters which are solid at 25°C; silicone waxes such as methyloctadecaneoxypolysiloxane and poly (dimethylsiloxy)

stearoxysiloxane; stearyl mono- and diethanolamide; rosin and its derivatives such as the abietates of glycol and glycerol; hydrogenated oils solid at 25°C; and sucroglycerides.

Thickeners (viscosity control agents) which may be used in effective amounts in aqueous systems include: algin; carbomers such as carbomer 934, 934P, 940 and 941; cellulose gum; cetearyl alcohol, cocamide DEA, dextrin; gelatin; hydroxyethylcellulose; hydroxypropylcellulose; hydroxypropyl methylcellulose; magnesium aluminum silicate; myristyl alcohol; oat flour; oleamide DEA; oleyl alcohol; PEG-7M; PEG-14M; PEG-90M; stearamide DEA; Stearamide MEA; stearyl alcohol; tragacanth gum; wheat starch; xanthan gum; and the like.

In the above list of thickeners, DEA is diethanolamine, and MEA is monoethanolamine.

Thickeners (viscosity control agents) which may be used in effective amounts in nonaqueous systems include, aluminum stearates; beeswax; candelilla wax; carnauba; ceresin; cetearyl alcohol; cetyl alcohol; cholesterol; hydrated silica; hydrogenated castor oil; hydrogenated cottonseed oil; hydrogenated soybean oil; hydrogenated tallow glyceride; hydrogenated vegetable oil; hydroxypropyl cellulose; lanolin alcohol; myristyl alcohol; octyldodecyl stearoyl sulfate; oleyl alcohol; ozokerite; microcystalline wax; paraffin; pentaerythrityl tetraoctanoate; polyacrylamide; polybutene; polyethylene; propylene glycol dicaprylate; propylene glycol dipelargonate; stearalkonium hectorite; stearyl alcohol; stearyl stearate; synthetic beeswax; trihydroxystearin; trilinolein; tristearin; zinc stearate; and the like.

Suitable film formers which may be used include: acrylamide/sodium acrylate copolymer; ammonium acrylates copolymer; Balsam Peru; cellulose gum; ethylene/maleic anhydride copolymer; hydroxyethylcellulose; hydroxypropylcellulose; polyacrylamide; polyethylene; polyvinyl alcohol; pvm/MA copolymer (polyvinyl methylether/ maleic anhydride); PVP (polyvinylpyrrolidone); maleic anhydride

copolymer such as PA-18 available from Gulf Science and Technology; PVP/hexadecene copolymer such as Ganex V-216 available from GAF Corporation; acrylic/acrylate copolymer; and the like.

Generally, film formers can be used in amounts of about 0.1% to about 10% by weight of the total composition with about 1% to about 8% being preferred and about 0.1% to about 5% being most preferred.

Preservatives which may be used in effective amounts include: butylparaben; ethylparaben; imidazolidinyl urea; methylparaben; O-phenylphenol; propylparaben; quaternium-14; quaternium-15; sodium dehydroacetate; zinc pyrithione; and the like.

The preservatives are used in amounts effective to prevent or retard microbial growth. Generally, the preservatives are used in amounts of about 0.1% to about 1% by weight of the total composition with about 0.1% to about 0.8% being preferred and about 0.1% to about 0.5% being most preferred.

Perfumes (fragrance components) and colorants (coloring agents) well known to those skilled in the art may be used in effective amounts to impart the desired fragrance and color to the compositions of this invention.

Other ingredients which may by added or used in amounts effective for their intended use include: biological additives to enhance performance or consumer appeal such as amino acids, proteins, vanilla, aloe extract, bioflavinoids, and the like; buffering agents; chelating agents such as EDTA; emulsion stabilizers; pH adjusters; opacifying agents; and propellants such as butane carbon dioxide, ethane, hydrochlorofluorocarbons 22 and 142b, hydrofluorocarbon 152a, isobutane, isopentane, nitrogen, nitrous oxide, pentane, propane, and the like.

The ingredients --sunscreening agents, mollients, emulsifiers, surfactants, solvents for sunscreening agents, waxes, thickeners, film formers, humectants, preservatives,

surfactants, perfumes, coloring agents, biological additives, buffering agents, chelating agents, emulsion stabilizers, opacifying agents, pH adjusters, and propellants-- are all well known to those skilled in the art, and the determination of which ingredients to use to obtain the intended formulations (lotions, creams, gels, sticks, and aerosols), and determination of the variations in the amounts which may be used to achieve the intended functions and effects of these ingredients are well within the capabilities of those skilled in the art without the need for undue experimentation. Further information may be obtained on these ingredients by reference to:

(1) Cosmetics & Toiletries, Vol. 102, No. 3, March 1987;

(2) Balsam, M.S., et al., editors, Cosmetics Science and Technology, 2nd edition, Vol. 1, pp 27-104 and 179-222 Wiley-Interscience, New York, 1972;

(3) Cosmetics & Toiletries, Vol. 104, pp 67-111, February 1989;

(4) Cosmetics & Toiletries, Vol. 103, No. 12, pp 100-129, December 1988; and

(5) Nikitakis, J.M., editor, CTFA Cosmetic Ingredient Handbrook, First Edition, published by The Cosmetic, Toiletry and Fragrance Association, Inc., Washington, D.C., 1988.

the disclosures of each being incorporated herein by reference thereto.

By using effective amounts of the exemplified components various types of cr ams, lotions, g ls, solid sticks,

and aerosol formulations can be blended in accordance with known compositions and procedures. In making the formulations it is preferred that the riboflavin, riboflavin phosphate or mixtures thereof not be subjected to heating or to high alkaline conditions. If PK-C Activators are used they should not be heated nor subjected to high alkaline conditions.

For example, a typical lotion formulation is listed in Table 1.

Table 1

TYPICAL LOTION FORMULATION

Ingredients	% By Wt. Range
Part 1	
Lanolin	0.2% - 1%
Cocoa Butter	2.0% - 5%
Emcol RHT (Glyceryl Stearate)[1]	2.0% - 4%
Hystrene 5016 (Stearic Acid)[2]	2.0% - 4%
Vitamin E Acetate	0.1% - 0.5%
Aloe Vera Lipo Quinone Extract	0.1% - 1.0%
Jojoba Oil	0.1% - 1.0%
Mineral Oil	1.0% - 7%
Propylparaben	0.1% - 1%
Medical Fluid 360 (Dimethicone)[3]	0.1% - 1%
Part 2	
Water	40% - 60%
Carbopol 941 (1%) (Polyacrylic Acid Polymer)[4]	10% - 35%
Propylene Glycol	2.0% - 7%
Triethanolamine 99%	0.1% - 3%
Lanogel 41 (PEG-75 Lanolin)[5]	0.25% - 1%
Methylparaben	0.1% - 0.5%
Sequestrene Na_2	0.01% - 0.08%
Part 3	
Perfume	0.01% - 0.5%

1 Witco Corp., Organics Division, NY, NY (also Witconol RHT)
2 Humko Chemcal, Memphis, Tenn.
3 Dow Corning Corp., Midland, Michigan
4 B.F. Goodrich Specialty Polymers and Chemical Division, Cleveland, Ohio
5 Amerchol Corp., Edison, NJ

To make the formulation listed in Table 1 parts 1 and 2 are heated separately to 180°F. Part 1 is then added to Part 2. The resultant blend is cooled to 120°F and Part 3 is then added.

A formulation containing riboflavin was prepared by combining the ingredients listed in Table 2.

Table 2

PRE TAN ACCELERATOR

Ingredients	% By Wt.
Part A	
Aloe Extract	0.25
Cetyl Alcohol	4.00
Myristyl Myristate	1.50
Propyl Paraben	0.10
Stearyl Alcohol	1.00
Part B	
Dimethicone, 350 cst*	0.60
Part C	
Glycerin, 99%	5.00
Methyl Paraben	0.20
dl-Panthenol	0.50
Sodium Lauryl Sulfate	0.40
Water, purified, USP	75.425
Part D	
Imidazolidinyl Urea	0.30
Riboflavin	0.20
Water	10.00
Part E	
Benzyl Alcohol	0.50
Felton Fragrance #332	0.025

* cst = centistokes (also abbreviated as "cs")

The ingredients in Part A and Part C (Table 2) were heated to about 77-82°C. Then Part A was stirred into Part C. Part B was

added to the mixture formed from Parts A and C. The resulting mixture of Parts A, C and B was force cooled to 65°C. The ingredients in Part D were dissolved together and then added at about 45-50°C to the mixture of Parts A, C, and B. To this resulting mixture there was added the ingredients of Part E at about 42-45°C, and the final mixture was mixed until the temperature of the mixture reached room temperature (about 25°C).

Examples of formulations which may prove useful which are oil-in-water creams, oil-in-water lotions, water-in-oil lotions, oil-in-water resistant creams and lotions, sticks, gels, oils and mousses may be found in, for example, Cosmetics & Toiletries, Vol. 102, pp 117-130, March 1987, the disclosure of which is incorporated herein by reference thereto. Examples of formulations which may prove useful which are hand and body lotions, oil-in-water emollient creams, moisturizing lotions, after sun emollient stick, facial spray mist, skin mousse and moisturizing gel may be found, for example, in Cosmetics & Toiletries, Vol. 102, pp 147-160, April 1987, the disclosure of which is incorporated herein by reference thereto. Those skilled in the art will appreciate that the formulations described in the above cited Cosmetics & Toiletries references (March and April 1987) represent types of formulations which may be suitably modified to allow for the addition of riboflavin, riboflavin phosphate or mixtures thereof, and that such modifications may be accomplished without the need for undue experimentation.

The following examples are illustrative only and should not be construed as limiting the invention in any way. Those skilled in the art will appreciate that variations are possible which are within the spirit and scope of the appended claims.

EXAMPLE 1

In this example the ability of riboflavin to enhance melanogenesis (tanning) was studied. Compositions containing riboflavin in amounts of 0.02% and 0.2% by weight of the total composition (vehicle and riboflavin) were studied. The vehicle was Coppertone After Tan Lotion, commercially available from Plough Inc., Memphis, TN. The vehicle composition would be similar to that given in Table 1 above.

Three groups of female Skh-2 pigmented hairless mice, five mice per group, were studied. Group 1 was the control and was treated with the vehicle only, Group 2 was treated with the 0.02% riboflavin composition, and Group 3 was treated with 0.2% riboflavin composition. Each group received topical treatment daily (Monday to Friday) to their dorsal surface (12 cm^2) of 2 $\mu l/cm^2$ of the appropriate vehicle or composition for four weeks. Irradiations were performed three times weekly (Monday, Wednesday and Friday) over the four week time period. The irradiations were performed using a 20 minute exposure each time from a bank of Kodacel 401-filtered FS-20 lamps. The mice were housed in a room lighted by F40GO gold fluorescent lamps. The test solutions were stored refrigerated in the dark when not in use.

After four weeks analysis was performed by taking histological sections from the midback of each mouse. DOPA stains were done on epidermal sheets and Warthin-Starry melanin stains were done on thin sections in accordance with procedures well known to those skilled in the art (see, for example, Luna, L., Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, McGraw-Hill Book Co., New York, 1968. Ten random fields on DOPA stained sections were counted at 100x magnification and a group mean calculated. Tests of melanocytes seen on Warthin-Starry sections were scored from +1 (a few melanocytes) to +4 (extensive

melanization) and a mean score per slide for each group calcuated. The results are given in Table 3.

Table 3

Group	DOPA (Group Mean)	Warthin-Starry (Mean/Slide)
Control (vehicle only)	78	34
0.02% riboflavin	110	33
0.2% riboflavin	188	86

EXAMPLE 2

The procedures of Example 1 were followed except that each group had 6 mice and the irradiations were conducted for three weeks. The results are given in Table 4.

Table 4

Group	DOPA (Group Mean)	Warthin-Starry (Mean/Slide)
Control	54.8	28.2
0.02% riboflavin	47.0	29.8
0.2% riboflavin	98.0	70.6

EXAMPLE 3

In this Example a study was conducted to determine the tanning enhancement efficacy of 0.2% riboflavin in Coppertone After Sun Lotion, (available from Plough Inc., Memphis, TN, the composition of the Lotion would be similar to that given in Table 1 above) on human subjects. The control was the Coppertone After Sun Lotion without riboflavin. The 0.2% riboflavin lotion was stored in a dark container to ensure photostability.

Six volunteers were selected with skin types I-III. Irradiation templates were devised with two circular holes, one inch apart, of 2 cm diameter each, providing two application sites of 12.57 cm^2. Each subject's arm was examined and templates were placed to expose sites on the lightly pigmented, hairless, inside forearm. Reference points outside the treatment area were marked with Castaderm for template alignment. The upper site, designated site I, was spaced approximately two inches from the inside bend of the elbow. The irradiation source was a bank of 4 Kodacel 401-filtered FS20 bulbs in a shuttered housing. The subject's skin was approximately 3 inches from the Kodacel filtered shutter. Both sites were irradiated simultaneously.

During week 1 of the study, the lotions were applied daily at 25 µl/site (2µl/cm^2) and air dried. During weeks 2 and 3, the applications were followed after a minimum of 2 hours by irradiations which increased daily in time until an MED (minimal erythermal dose) was achieved. After each subject's MED was reached, that exposure was continued to the end of the study. Erythema/tanning were scored visually during weeks 2 and 3 according to the following scale:

0 = no difference from surrounding skin
1 = slight erythema (pink)
2 = erythema (red)
3 = very slight tan

4 = tan
5 = medium brown tan
6 = dark brown tan

A "+" or "-" was used to indicate differences not one full grade apart.

Results are set forth in Tables 5 and 6. In Tables 5 and 6, I indicates Site I which was treated with the lotion containing 0.2% riboflavin, II indicates Site II which was the control, and column "C" gives the results of the comparison of Sites I and II indicating which site is darker as scored by the investigator.

TABLE 5
Visual Scoring of Tanning/Erythema

Irrad. Day	Subject 1			Subject 2			Subject 3		
	I	II	C	I	II	C	I	II	C
1	Memorial Day -			--	--	--	--	--	
2	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0
--	--	--	--	--	--	--	--	--	--
--	--	--	--	--	--	--	--	--	--
8	0	0	0	0	0	0	0	0	0
9	3	3	I	0	0	0	0	0	0
10	3	3	I	0	0	0	0	0	0
11	4	3	I	0*	0*	0	3	3	0
12	3	3	I	0*	0*	0	3	3	I
--	--	--	--	--	--	--	--	--	--
--	--	--	--	--	--	--	--	--	--
15	4	3	I	3	3	0	3	3	I
16	4	3	I	dropped out			3	3	II
17	4	3/1-	I				3	3	I
18	4/1	3/1	I				3	3	I
19	5	3	I				3	3	0
--	--	--	--				--	--	--
--	--	--	--				--	--	--
20	4	3	I				3	3	0
Darkest Site:	I						Equal		

* Freckles Appeared

TABLE 6
Visual Scoring of Tanning/Erythema (cont.)

Irrad. Day	Subject 4			Subject 5			Subject 6		
	I	II	C	I	II	C	I	II	C
1	--	--	--	--	--	--	--	--	--
2	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0
--	--	--	--	--	--	--	--	--	--
--	--	--	--	--	--	--	--	--	--
8	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0
11	0*	0*	0	0	0	0	1	1-	I
12	0*	0*	0	3	0	I	1-	0	I
--	--	--	--	--	--	--	--	--	--
--	--	--	--	--	--	--	--	--	--
15	dropped out			4	3	I	4/1	3	I
16				4-	3	I	4/1	3	I
17				3/1	3-	I	4/1+	3	I
18				4/1-	3	I	3/2	3/1	I
19				4	3	I	4/1	3	I
--				--	--	--	--	--	--
--				--	--	--	--	--	--
20				4	3-	I	3+/1-	3	I
Darkest Site:					I			I	

* Freckles Appeared

EXAMPLE 4

Using the same experimental procedure set forth in Example 2, the effects of 0.01% (by weight) DOPA phosphate (mixture of Isomers II and III, see U.S. 4,508,706) were compared to a combination of 0.01% (by weight) DOPA phosphate (mixture of Isomers II and III) and 0.02% (by weight) riboflavin, and to a combination of 0.01% (by weight) DOPA phosphate (mixture of Isomers II and III) and 0.2% by weight riboflavin. All lotions were stored refrigerated in the dark. The results are given in Table 7.

Table 7

Group	DOPA (Group Mean)	Warthin-Starry (Mean/Slide)
Control*	54.8	28.2
0.01% DOPA phosphate	86.4	58.2
0.02% Riboflavin*	47.0	29.8
0.20% Riboflavin*	98.0	70.6
0.01% DOPA phosphate and 0.02% Riboflavin	92.4	56.8
0.01% DOPA phosphate and 0.20% Riboflavin	177.6	89.2

* Same as in Example 2

In an earlier experiment of Example 4 the group responding with the greatest pigmentation increase (melanocyte count) over the control group was the group treated with 0.01% DOPA phosphate (mixture of Isomers II and III) plus 0.2%

riboflavin. However, the response in the earlier experiment could not be shown to be statistically different from the response due to 0.2% riboflavin alone. This may have been due to the degradation of DOPA phosphate Isomer II since the lotion was not stored refrigerated in the earlier experiment. The difference in the results set forth in Table 7 are statistically significant.

Those skilled in the art will appreciate that the total amount of all ingredients (components) used in the compositions of this invention equals 100% by weight of the total composition. Also, unless stated otherwise all percents and amounts are percent by weight of the total composition.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention and all such modifications are intended to be included within the scope of the claims.

WHAT IS CLAIMED IS:

1. A composition comprising riboflavin, riboflavin phosphate or mixtures thereof in an amount effective to enhance melanin production when said composition is applied topically to the skin.

2. The composition of Claim 1 wherein said composition comprises about 0.1% to about 2% by weight of the total composition of riboflavin, riboflavin phosphate or mixtures thereof.

3. The composition of Claim 2 wherein said riboflavin, riboflavin phosphate or mixtures thereof are encapsulated in liposomes.

4. The composition of Claim 2 wherein said composition comprises an effective amount of water and an effective amount of a humectant.

5. The composition of Claim 1 wherein there is added to said composition an effective amount of at least one other ingredient selected from the group consisting of: Protein Kinase C Activators, DOPA phosphates, sunscreening agents, emollients, emulsifiers, solvents for sunscreening agents, waxes, thickeners, film formers, humectants, antioxidants, preservatives, surfactants, skin penetration enhancers, perfumes, biological additives, buffering agents, chelating agents, emulsion stabilizers, opacifying agents, pH adjusters, propellants and coloring agents.

6. The composition of Claim 2 wherein there is added to said composition an effective amount of at least one other ingredient selected from the group consisting of Protein Kinase C Activators, DOPA phosphates, and sunscreening agents .

7. The composition of Claim 6 wherein the Activator is selected from the group consisting of: diacylglycerols, triacylglycerols, lipopolysaccharides, unsaturated free fatty acids, saturated short chain free fatty acids, glycerolphospholipids, enzymes which hydrolyze glycophospholipids to diacylglycerols, and bryostatins.

8. The composition of Claim 6 wherein diacylglycerol is added to said composition.

9. The composition of Claim 8 wherein said diacylglycerol is selected from the group consisting of:

(a) 1,2-dioctanoyl glycerol;
(b) 1,2-didecanoyl glycerol;
(c) 1-oleoyl-2-acetyl glycerol;
(d) 1-acetyl-2-oleoyl glycerol;
(e) 1,2-dihexanoyl glycerol;
(f) 1-stearyl-2-arachidonyl glycerol;
(g) 1-stearyl-2-oleoyl glycerol;
(h) 1,2-dipalmitoyl glycerol;
(i) 1,2-distearyl glycerol;
(j) 1,2-dioleoyl glycerol;
(k) diarachidonin;
(l) diolein;
(m) dipalmitin; and
(n) distearin.

10. The composition of Claim 8 wherein said diacylglycerol is a diacyl-sn-glycerol.

11. The composition of Claim 10 wherein said diacylglycerol is 1,2-dioctanoyl-sn-glycerol.

12. A method of enhancing melanin production comprising applying topically to the skin a composition of any of Claims 1-11.

13. The use of a composition of any of Claims 1-11 to manufacture a composition for enhancing melanin production when applied topically to the skin.

14. The use of a composition of any of Claims 1-11 to enhance the production of melanin when said composition is applied topically to the skin.

15. A process for producing a composition for enhancing melanin production when said composition is applied topically to the skin comprising combining riboflavin, riboflavin phosphate or mixtures thereof with suitable solvents.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 90/06328

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: A 61 K 7/42, A 61 K 7/48

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	FR, A, 2096712 (GIRAUX) 25 February 1972 see page 1, lines 15-17; page 3, lines 22-29; page 5, lines 18-32; claims --	1-2,5,12-15
X	CH, A, 642537 (TUR) 30 April 1984 see the whole document --	1-2,4-6, 12-15
X	FR, A, 2624374 (INDUCHEM AG) 16 June 1989 see claims 1,9-10,12,14; examples 2-4 --	1-6,12-15
X,P	EP, A, 0386680 (PLOUGH, INC.) 12 September 1990 see claims 1-15 --	1-5,12-15

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
31st January 1991	14. 02. 91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	miss T. MORTENSEN

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	Handbuch der Kosmetika und Riechstoffe, 1973, Dr. Alfred Hüthig Verlag, (Heidelberg, DE), H. Janistyn: "Photosensibilatoren", pages 743-745 see page 743	1,12-15

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9006328 SA 41997

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 11/02/91
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
FR-A- 2096712	25-02-72	None		
CH-A- 642537	30-04-84	None		
FR-A- 2624374	16-06-89	CH-A-	675967	30-11-90
		AU-A-	2578488	15-06-89
		DE-A-	3836849	22-06-89
		GB-A-	2213376	16-08-89
EP-A- 0386680	12-09-90	AU-A-	5195090	09-10-90
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